Microspectrofluorometry on Fly Photoreceptors In Vivo. Spectral Properties of Visual Pigment Fluorescence

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Published in:
Vision Research

DOI:
10.1016/0042-6989(84)90352-3

RHODOPSIN–PORPHYROPSIN SYSTEM IN CRAYFISH

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A sensitive analytical method was developed to study the rhodopsin-porphysopsin system in the eye. Oximes of 11-*cis*-retinal, 11-*cis*-3-dehydroretinal, all-*trans*-retinal, and all-*trans*-3-dehydroretinal were quantitatively determined by high-pressure liquid chromatography. The rhodopsin and porphyropsin of bullfrog retina were analyzed with this method. The results agreed with those obtained from the bleaching kinetics of visual pigments extracted with detergent. The analytical method was so sensitive that a 1/500 part of a bullfrog retina was sufficient for analysis. We studied the chromophore of the visual pigment of freshwater crustacean with this analytical method. The dark-adapted eye of crayfish (*Procambarus clarkii*) contained 11-*cis*-3-dehydroretinal which was isomerized to all-*trans* form by light-adaptation. All-*trans*-isomers produced by light were slowly reconverted to 11-*cis*-isomers in the dark with a half-time of 20 h (20°C). Seasonal variation was observed in the 3-dehydroretinal content. The value was below 5% in summer and about 40% in winter. The results suggest that the crayfish has a rhodopsin-porphyropsin system similar to that in freshwater fish.

LOCALIZATION OF DIFFERENT VISUAL PIGMENTS WITHIN THE RHABDOMS OF INSECT COMPOUND EYES

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After identification of visual pigments in extracts from insect eyes, there remains the problem of their distribution between the different types of visual cells within the ommatidium. Compound eyes after illumination with monochromatic light were investigated by microspectrophotometry and electron microscopy. Photometrically, difference spectra could be obtained from single rhabdomeres of "open" rhabdoms, indicating only one receptor pigment within a rhabdomere. In fused rhabdoms, distinct changes of the microvillar structure were found in the rhabdomeres of different receptor cells after different monochromatic illuminations. The changes were proved to occur *in vivo* only temporarily. Examples presented are the eyes of hemiptera, especially of the pond skater, *Gerris lacustris*, with three pigments, and of nocturnal lepidoptera, especially of the armyworm moth, *Spodoptera exempta*, with four pigments.

This work was done in collaboration with Drs B. Hamann, C. C. Meinecke, P. Schlecht, B. Welsch (Bochum), and Dr R. Schwind (University of Regensburg). It was financially supported by Deutsche Forschungsgemeinschaft.

MICROSPECTROFLUOROMETRY ON FLY PHOTORECEPTORS *IN VIVO*. SPECTRAL PROPERTIES OF VISUAL PIGMENT FLUORESCENCE

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Visual pigment fluorescence has been studied in the compound eyes of houseflies *Musca* and blowflies *Calliphora*, white eyed mutants, *in vivo*. Rhodopsin R fluorescence is negligible, but the metarhodopsin state M fluoresces distinctly in the red. At extreme illumination intensities a 3–5 times stronger fluorescing M′ state is populated. Both M and M′ have emission spectra, peaking at about 660 nm, which do not noticeably change in the physiological temperature range, so, indicating the rigidity of the visual molecule in the membrane. The fluorescence life times of M and M′, measured by applying a nitrogen pulse laser, are shorter than 40 nsec. Metarhodopsin M fluorescence has been used in a study of the photochemical cycle. The results were in good agreement with those obtained by transmission microspectrophotometry [Kruizinga *et al.* (1983), *Biophys. Struct. Mech.* **9**, 299]. Microspectrofluorometry hence can be utilised for analysing photopigment processes in completely intact, living animals.